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Pollination quantity and quality in relation to plant population size, flower diversity and flower constancy of syrphids in the devil's bit scabious *Succisa pratensis* (Dipsacaceae)

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SUMMARY

The effect of population size and the biotic environment (i.e. a low or a high diversity of other flowering plants) on visitor guild, visitation rate and pollen deposition are analysed in nine Dutch *Succisa pratensis* populations in 2002 and 2003. Insect species frequencies and visitation rate, flower constancy as visitation sequences and as body pollen-load composition, pollen composition of stigmatic loads and the number of *S. pratensis* pollen per stigma were measured. Between 5 and 20 insect species per population and 34 in total were visitors of *S. pratensis*. Twelve insect species contributed to >75% of the visits to *S. pratensis*, most of which were syrphids and bumblebees. In 2002, 1.4% of the total number of visits and in 2003, 29.5% were made by bumblebees. The visitation rate varied between 5 and 18 visits per *S. pratensis* head per hour. Large populations of *S. pratensis* were visited by more insect species, but visitation rates did not differ between *S. pratensis* populations. Syrphid species differed in their flower constancy, with *Helophilus pendulus* being a less flower-constant species compared to *H. trivittatus* and *Eristalis horticola*. In small populations the average syrphid load contained between 23 and 18.5% *S. pratensis* pollen, in large populations between 46.5 and 54%. The body loads contained also pollen of plant species from at least 300 m away from *S. pratensis* populations. The percentages of conspecific pollen on stigmas was less (60%) in smaller than in larger populations (70 - 100%). Stigmas had received between 5 and 17 pollen grains at the end of the day, small populations had received significantly less conspecific pollen than large populations. Potential seed set varied between 65% (small populations) and 94% (large populations) of the flowers per head.

The quality of pollination and not the number of visits in large populations was more favourable for seed set than in small populations, with a strong effect of population size and much less of the diversity of the environment. If heterospecific pollen deposition leads to a lower seed set, then small populations in a diverse environment will have the lowest percentage of viable seeds per head, and large populations in an environment with no other simultaneously flowering species will have the highest percentage of seed set.

Andrena marginata, a specialist bee foraging on *S. pratensis*, is extinct in the Netherlands. If it was a better pollinator than syrphids and bumblebees, then the shift to a more generalist pollinator assemblage of only syrphids and bumblebees had negative consequences for the pollination of *S. pratensis*. There may be a positive feedback of declining plant populations, affecting the abundance and efficiency of pollinators, thereby speeding up the decline of the plant population. The abundance or absence of specialist pollinators can be good indicators of declining plant population sizes.

INTRODUCTION

Both plants and animals are decreasing in number of species and in population size, as a result of human impact. The vulnerability of plants to extinction is somewhat hidden by the fact that plants may survive for many years without recruitment (Oostermeijer *et al*. 1992) and by the fact that a population may persist as seed in the seed bank although adult plants have disappeared (Bekker *et al*. 1999). Insects, acting as herbivore or pollinator, are supposed to be faster indicators of declining plant population size, because they depend on the green parts or the flowers of plants (Kevan 1999). In case of pollinators, there may be even a risk of a positive feedback: a declining plant population size may affect the abundance and efficiency of pollinators, and this change in turn may speed up the decline of the plant population. To further explore this idea, we need detailed information about the complex relationship not only between plant population size and the behaviour of pollinators, but also about the effects of associated flowering plants on the efficiency of this relationship.

Insects that confine their menu choice to one or few plant species are called specialists; insects foraging on several or many plant species are called generalists. These specialists are thought to be more sensitive to habitat deterioration than generalist insects, because interactions with their host plants will be lost (Olesen & Jain 1994) and the insects have no alternatives. The specialist bee species *Andrena marginata* forages exclusively on species of the family of Dipsacaceae (Westrich 1990), with *Succisa pratensis* as main pollen source and *Scabiosa columbaria* as one of the alternatives in the Netherlands (Peeters *et al*. 1999). Both plant species are decreasing rapidly in the Netherlands; *Succisa pratensis* is classified as a "sensitive species" (from >10,000 1x1 km squares in 1935 towards 1000-3000 in 1995; Tamis *et al*. 2004), and *S. columbaria* is considered as an "endangered species" (from 300-1000 1x1 km squares in 1935 towards 31-100 in 1995). The bee species used to be rather common in the eastern part of the Netherlands, but in 1962 it was observed for the last time (Peeters *et al*. 1999). Data of other insect species as flower visitors of the two plant species in the past are scarce. Knuth (1898) mentioned 37 insect species visiting *S. pratensis* in north and central Germany, with most species belonging to the Hymenoptera (40.5%). Proctor *et al*. (1996) especially mentioned *A. marginata* with *S. pratensis* as one of its favourites.

Flowers of many plant species are visited by more than one insect group and by even more insect species (see for instance Herrera 1987; Petanidou 1991; Waser *et al*. 1996; Johnson & Steiner 2000; Keys *et al*. 2005). Visiting insects may differ in the efficiency of pollen collection from the anthers and pollen deposition on the stigmas. An estimate of effectiveness of visitors for pollination of the whole plant population can be made from various perspectives (see also Waser & Price 1990): the number of visits spent by insect groups, and several foraging characteristics important for the efficiency of visitation of the two main groups, e.g. foraging speed (the number of flowers and flower heads visited per minute), and body pollen loads (the number of pollen grains on the insects). Flowers may also receive visits from insect species that visit other plant species (generalists) as well, and of insect species or individuals that confine their visits to one or a few related species

(specialists) (Jordano 1987; Bronstein 1995; Waser *et al*. 1996; Memmott 1999; Olesen & Jordano 2002). Because flowers of many plant species are visited by a variety of insects, often including both generalists and specialists, the disappearance of a specialist insect does not need to be a catastrophe for the plant species under concern. However, the pollen load deposited on stigmas by specialists may contain more conspecific pollen grains than that deposited by generalist insects. Thus the quality of the pollination may change from a more or less pure stigmatic pollen load to a more heterospecific stigmatic load if the visitor guild changes from one containing an important contribution of specialists towards one with more generalists. Only a few studies include both pollinator abundance and effectiveness. These studies suggest that decoupling of pollinator abundance (quantity aspect) and pollinator quality occurs frequently among insect-pollinated plants (Sugden 1986; Schemske & Horvitz 1989; Pettersson 1991; Mayfield *et al*. 2001). Olsen (1997) found that pollinator importance, calculated as the product of pollination efficiency and relative abundance, was dictated by a pollinator's relative abundance. In his study, insects differed up to three times in pollination efficiency.

In this paper we analyse the effect of population size and the biotic environment, i.e. a low or a high diversity of other flowering plants, on visitor guild, visitation rate and pollen deposition in populations of *Succisa pratensis*. We assume that in a large population insects will be more flower-constant, either passive or active, so stigmatic loads in large *S. pratensis* populations will contain more conspecific pollen grains than in small populations. A diverse environment invites insects to visit more plant species during a single foraging trip. Furthermore, we relate frequencies of insect species and visitation rates to the number of conspecific pollen on the stigmas.

MATERIAL AND METHODS

The plant

Succisa pratensis Moench (devil's bit scabious, Dipsacaceae), a perennial herb, grows occasionally in unfertilised hay fields and along roadsides in the Netherlands. It is a characteristic species of biodiverse, slightly acidic grasslands (Nardetea) (Vergeer *et al*. 2003b). Changes in land use, habitat fragmentation and deterioration have reduced its distribution area by 50-75% during recent decades (van der Meijden *et al*. 2000). Vergeer *et al*. (2003a) showed that population size is indeed strongly influenced by habitat quality. The remaining populations are isolated from each other and many are very small. The main flowering season in the study area starts in August and continues till mid October. The plant forms 1-10 flower branches, with 1-20 flower heads each. The blue-violet flowers (3 mm long) are arranged in hemispheric flower heads, with on average 60 flowers per head (diameter 2-3 cm). The flowers are protandrous, first presenting four anthers (in a sequence of two groups of two). The first flowers are displayed in two crowns at the bottom and near the top of the flower head. Both anthers and styles protrude out of the small, tubular flower. Both male and female flowers produce nectar at a rate during day time of 0.029 μl per hour with a sugar concentration of 14.1% (M.M. Kwak, unpubl. data).

Populations

Data were collected in nine populations in the north of the Netherlands in 2002 and 2003. Five populations were located in road verges near Assen (De Haar, Gasteren, Ekehaar, Eleveld and Annen, in the province of Drenthe), three populations in nature reserves (Wijnjewoude and Rotstergaast, in the province of Fryslân; and Reitma in the province of Drenthe). One population was artificially created in 1991 (Assen). In this chapter only data collected in the month September are presented to minimise the variation in phenology of insects and plants. *Succisa* populations differed in number of flowering heads (called population size) and the number of simultaneously flowering co-occurring plants. On each observation day the total number of flowering heads at that moment was counted and used as population size. Population characteristics are mentioned in table 7.1.

Insect observations

Species frequencies and visitation rate

Insect observations were made in 2002 and 2003, between 10.00 and 16.00 h. In each population once per observation day, the number of individuals per species of insects visiting *S. pratensis* flowers were counted in a transect of ca. 100m length, depending the population size. In addition, in a plot with a known number of flower heads (35), insect visits were scored during ten minutes. This was done between two and four times per day per population.

Flower constancy: visitation sequences

The flower constancy of three syrphid species that visited *Succisa* flower heads most frequently was analysed (in 2002). Flower constancy is a quality component of pollination (when an insects are highly constant, then a pure pollen deposition load is expected). Following of insects was done in three populations: Ekehaar, Wijnjewoude and Annen. Observations always started with a visit to a *S. pratensis* head. The amount of changing was calculated by dividing the number of intraspecific transitions by the total number of transitions. This index ranges from 0 to 1 and the outcome is the proportion of intraspecific transitions (Slaa & Biesmeijer 2003). The minimum bout length for analysing was 5 visits, which equals four transitions.

Flower constancy: body pollen-load composition

Flower constancy over a longer period of time can be derived from the composition of the pollen load on the body of insects. Although syrphid flies spend time in cleaning their body and eating pollen, they often carry a reasonable amount of pollen on their body. In order to detect differences in flower constancy between four common syrphid species, pollen body loads were analysed. Ten individuals per species were sampled. Insects were captured after the observation that they had visited a head of *S. pratensis*; resampling was prevented. Insects were slightly anaesthetised with CO_2 and the ventral side of the body (this parts may contact *S. pratensis* stigmas) was cleaned with a piece of a sticky gel (Beattie 1972). A microscope slide was made by melting the gel and at least 300 pollen grains

Table. 7.1. Characteristics of the *Succisa pratensis* populations under investigation. Population size is given between brackets as maximum of the counted number of flowering heads during the observation period. Number of simultaneously flowering plant species is given between brackets. Observations are made on 1. frequencies of visiting insects and visitation rates; 2. flower constancy; 3. composition of pollen loads on insect bodies; 4. composition of stigmatic loads; 5. number of *S. pratensis* pollen grains per stigma. Observation 1 is made in 2002 and 2003, 2 and 3 only in 2002, 4 and 5 only 2003.

Population	coordinates	maximum population size	Number of simultaneously flowering plant species	observations
De Haar	52°58'N, 6°32'E	small (135)	low (5)	1
Eleveld	52°57'N, 6°34'E	small (160)	low (7)	1, 4, 5
Gasteren	52°02'N, 6°40'E	small (250)	low (4)	1, 3, 4, 5
Ekehaar	52°56'N, 6°37'E	small (350)	high (17)	1, 2, 3
Reitma	52°53'N, 6°40'E	large (6000)	low (2-3)	1, 4, 5
Wijnjewoude	52°03'N, 6°10'E	large (>10,000)	low (2)	1, 2, 3
Rotstergaast	52°55'N, 5°56'E	large (27,000)	low (3)	1
Annen	53°04'N, 6°41'E	large (1,600)	high (9-16)	1, 2, 3, 4, 5
Assen	52°59'N, 6°35'E	large (1,750)	high (30)	1

were counted and identified under a light microscope (10 x 10 or 10 x 40 magnification). A reference pollen collection of flowering species in the environment was prepared in the same way. In order to detect effects of population size and diversity of the flowering environment on the composition of the body load, two syrphid species were sampled in four populations.

Plant observations

Pollen composition of stigmatic loads

The overall result of foraging of all insects is pollen deposition on stigmas. Before the start of the observations, heads were checked with a hand magnifier of 15x for virginity and receptivity (only in 2003). Heads were allowed to be visited during 5-6 hours during the day. After this period, stigmas (from seven heads per population, five populations) were cleaned and microscope slides were prepared in the same way as was done with the insects. If possible, 300 pollen grains were counted and identified; the percentage of *S. pratensis* was calculated.

Number of *S. pratensis* pollen per stigma

Virgin female heads that will become receptive that day were marked before observations started (only in 2003). At the end of the day (an exposure period of 5-6 hours) the number of pollen grains per stigma (n= 15 per head) was counted with a magnifier. Seven heads per population were counted. For seed set not only the mean number but also the distribution of the grains over the stigmas is important. Often a surplus of pollen is needed

for the fertilisation of one ovule (Waser & Fugate 1986). We used the same minimum number of grains as is needed in the related species *Scabiosa columbaria* (Velterop 2000): four. We calculated the potential seed set: the percentage of flowers per flower head that had more than four pollen grains per stigma.

Statistics

The effect of population size and flower species richness on the number of visitor species, purity of pollen deposition on stigmas, the number of deposited pollen and potential seed set were analysed with multiple linear regression followed by a simple curve-fit analysis. Changing indices of insects were tested for significance within and between populations with Kruskal-Wallis tests, with Mann-Whitney tests for differences between groups. Body loads of insects were tested for significance within and between populations with univariate analyses of variance (ANOVA) with Tukey-tests as post hoc test for differences between groups. The effect of population type on visitation rate, purity of pollen deposition on stigmas, the number of deposited pollen and potential seed set were analysed with univariate ANOVA with Tukey-tests as post hoc test for differences between groups. All statistical tests were performed with the package SPSS for Windows version 12.0.1 (2003).

RESULTS

Insect observations

Species frequencies and visitation rate

In 2002, 21 insect species visited *S. pratensis* (transect observations, fig. 7.1); the large majority of visits were from syrphid flies (96.2%). In 2003, 23 species were observed (plot observations), but the proportion of syrphid species and visits (53.9%) was lower than in 2003, and those of bees (almost exclusively bumblebees) was higher (32.7%, fig. 7.1). The contribution of insect species per population and per day to reach 75% of all visits was calculated and the frequency of being one of the contributors was scored. The frequency of these scores is shown in figure 7.2. Twelve species contributed to 75% of the visits on at least one day in one population. *Eristalis tenax, H. trivittatus* and *B. pascuorum* can be considered as the most important visitors for *S. pratensis*. The number of species contributing to 75% was larger in 2003 than in 2002 (10 vs. 7), and the years differ in bee and syrphid species.

The population size of *S. pratensis* affected the number of visitor species positively, while the flower diversity of the environment had no influence on the insect species composition of *S. pratensis*. The relation between *S. pratensis* population size as a continuous variable and the number of visitor species was best described with logarithmic function ($r^2 = 0.69$, fig. 7.3). There was no relation between flower diversity as a continuous variable and the number of visitor species.

In 2002, small populations had fewer visitor species than only one large population (11, 6 and 9 vs. 18 and 9) for all days combined (fig. 7.3). Small populations had *Eristalis horticola, E. tenax, Helophilus pendulus* and *H. trivittatus* as most frequently occurring

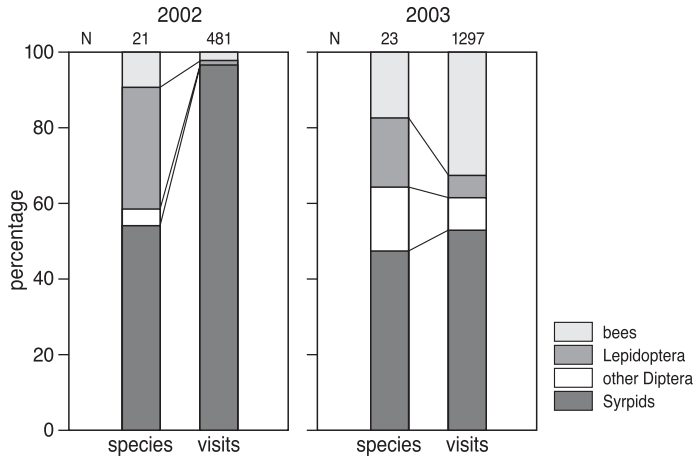

Fig. 7.1. Proportions of four main insect taxonomical groups as percentage of all species and the percentage visits brought by the insect groups for *Succisa pratensis* in 2002 and 2003. The N-values are the number of species (left bars within years) or visits (right bars). The lines between the bars indicate the difference of the relative contribution of the taxonomical groups to the number of species and the number of visits.

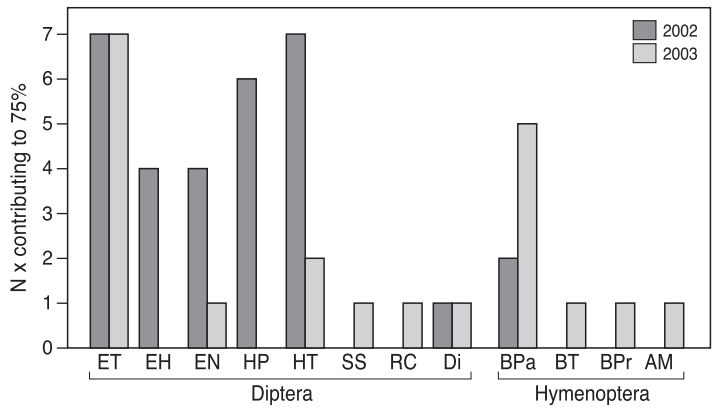

Fig. 7.2. Insect species contributing to 75% of visits on *Succisa pratensis* in 2002 and 2003. The contribution of insect species per population and per day to reach 75% of all visits was calculated, and the frequencies of these scores are shown as bars. ET= *Eristalis tenax*, EH= *E. horticola*, EN= *E. nemorum* and *E. arbustorum*, HP= *Helophilus pendulus*, HT= *H. trivittatus*, SS= *Sericomyia silentis*, RC= *Rhingia campestris*, Di= other Diptera, BPa= *Bombus pascuorum*, BT= *B. terrestris*, BPr= *B. pratorum*, and AM= *Apis mellifera*.

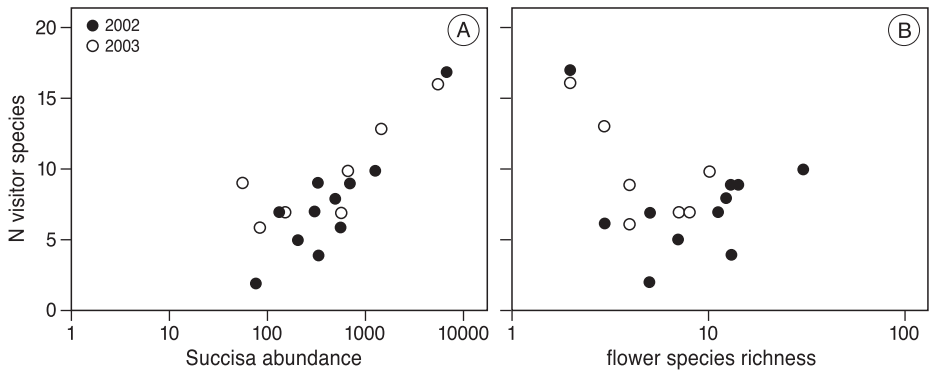

Fig. 7.3. Effect of the abundance of *S. pratensis* (A) and flower species richness (the number of other flowering species in the environment, B) on the number of flower visiting insect species on *Succisa pratensis*. There was a positive effect of population size ($r^2 = 0.69$), but no significant effect of flower species richness. Both x-axes are log-scaled. The data points are shown for the two years separately, but r^2-values are based on all data together.

species. Large populations had the same species, but also small *Eristalis* species (*E. nemorum* and *E. arbustorum*). In one small population (Ekehaar), *Bombus pascuorum* was an important visitor on one day. In 2003, the two small populations had fewer visiting species than the two large populations (10 and 7 vs. 20 and 14) for all days (fig. 7.3). The most important visitors in the small populations were bumblebees (*B. pascuorum*) and syrphids (*E. tenax*), in the two large populations mainly syrphids (*E. tenax*), and in the large population Reitma also bumblebees (*B. pascuorum*).

The mean number of visits received per *S. pratensis* flower head varied in 2002 between 5 and 13 visits per hour per day; in 2003 between 5 and 12. In both years the visitation rates did not differ significantly between population types either size or diversity of the environment (2002: $F_{3,33} = 2.4$, $p > 0.05$; 2003: $F_{3,20} = 0.73$, $p > 0.5$; fig. 7.4).

Flower constancy: visitation sequences

The observed syrphid species showed a flower constancy between 0.74 and 1.0, which means that most of the visited flowers were *S. pratensis* (table 7.2). Only *Helophilus pendulus* did react significantly concerning population type (diversity of the environment): in the populations with a high flower diversity its flower constancy was lower than in populations with a low flower diversity ($X^2 = 13.3$, $p < 0.005$). *Helophilus pendulus* was more constant in a larger *S. pratensis* population with a low plant diversity (0.99), and it tended to be intermediate in the large population with a high flower diversity (0.86), but did not differ significantly from the small population (0.74). Flower constancy varied only for *H. pendulus* but not the other two syrphid species, that always had constancies larger than 0.90. The species differed significantly in population small-high ($X^2 = 6.9$, $p < 0.05$; table 7.2). Overall, the flower constancies of the three species are 0.86, 0.96 and 0.95 respectively (data derived from table 7.2).

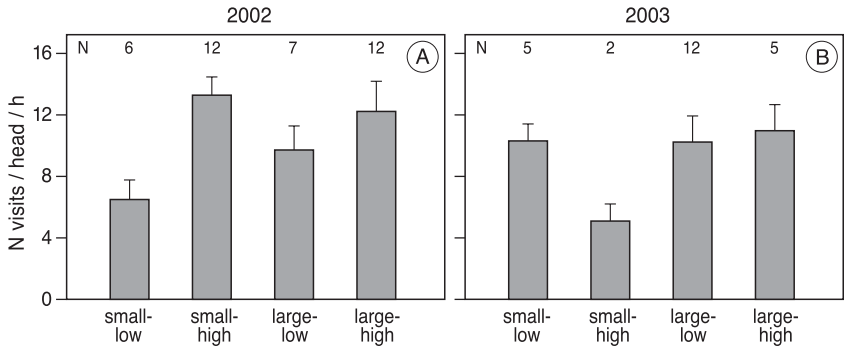

Fig. 7.4. Visitation rates on *Succisa pratensis* patches at four population types, based on flower abundance of *S. pratensis* (small or large populations) and the number of simultaneously flowering plant species (low or high diversity) in 2002 (A) and 2003 (B). No significant differences were found between populations types in any of the years.

Table. 7.2. Flower constancies (mean ± SE) of three syrphid species as frequent visitors of *Succisa pratensis* in populations differing in number of flowering heads of *S. pratensis* (small, large) and other flowering species (low, high). The populations were Ekehaar, Wijnjewoude and Annen. Flower constancy is the number of intraspecific transitions per plant species divided by the total number of transitions. The number of sampled individuals are between brackets. Significant differences between species are indicated with different capital letters, between the populations with small letters, n.a. means no value available.

	Population type (size *S. pratensis*, diversity flowering plants)		
Syrphid species	small, high	large, low	large, high
Helophilus pendulus	0.74 ± 0.09 (7)^{Aa}	0.99 ± 0.01 (18)^{b}	0.86 ± 0.06 (9)^{a}
Helophilus trivittatus	0.95 ± 0.03 (17)^{B}	1.0 ± 0 (4)	0.94 ± 0.03 (11)
Eristalis horticola	0.95 ± 0.02 (14)^{B}	n.a.	0.94 ± 0.03 (11)

Flower constancy: body pollen load composition

Syrphid flies carried loads that contained between 20 and 78% *S. pratensis* pollen (table 7.3), and between 24 and 508 *S. pratensis* pollen grains (table 7.4). Not all syrphid species could be sampled in all populations, making comparisons between species difficult. Differences between the species were found for the percentage of *Succisa* pollen at population large-low, where *H. pendulus* had a higher fraction *Succisa* pollen grains than the other species ($F_{3,36}$= 7.1, p< 0.005, table 7.3); for the absolute number of pollen in the populations small-high ($F_{1,18}$= 11.8, p< 0.005) and large-high ($F_{2,29}$= 18.3, p< 0.001), where *E. horticola* carried five to seven times more *S. pratensis* pollen grains than the other syrphid species (table 7.4).

Table. 7.3. Percentages of *Succisa pratensis* pollen grains relative to other plant species in body loads of four syrphid species in four *S. pratensis* populations. The number of sampled individuals are between brackets. Significant differences between species are indicated with different capital letters, between the populations with small letters, n.a. means no value available.

	population type (*S. pratensis* size, diversity of flowering plants)			
Syrphid species	small, low	small, high	large, low	large, high
Helophilus pendulus	23 ± 7 (10)^{a}	20 ± 5 (10)^{a}	78 ± 5 (10)^{Ab}	54 ± 9 (9)^{b}
Helophilus trivittatus	n.a.	n.a.	38 ± 10 (10)^{B}	40 ± 9 (11)
Eristalis horticola	n.a.	37 ± 7 (10)^{a}	45 ± 10 (10)^{Bab}	67 ± 8 (11)^{b}
Eristalis tenax	n.a.	n.a.	24 ± 9 (10)^{B}	n.a.

Table. 7.4. Number of Succisa pratensis pollen grains in body loads of four syrphid species in four S. pratensis populations. The number of sampled individuals are given between brackets. Significant differences between species are indicated with different capital letters, between the populations with small letters, n.a. means no value available.

	population type (*S. pratensis* size, diversity of flowering plants)			
Syrphid species	small, low	small, high	large, low	large, high
Helophilus pendulus	95 ± 51 (10)	49 ± 11(10)^{A}	66 ± 19 (10)	102 ± 27 (9)^{A}
Helophilus trivittatus	n.a.	n.a.	27 ± 6 (10)a	105 ± 24 (11)^{Ab}
Eristalis horticola	n.a.	341 ± 84 (10)Bab	96 ± 51 (10)a	508 ± 85 (11)^{Bb}
Eristalis tenax	n.a.	n.a.	24 ± 9 (10)	n.a.

The loads of *H. pendulus* were more pure in large than in small populations (large populations: 54-78 %; small populations 20-23 % *S. pratensis* pollen grains; $F_{3,35}= 17.4$, $p< 0.001$; table 7.3). For the absolute number of pollen grains there was a similar tendency, but this was not significant (table 7.4). Also the loads of *Eristalis horticola* contained a larger proportion of *S. pratensis* pollen grains in large than in small populations ($F_{2,28}= 3.4$, $p< 0.05$; table 7.4). However, the difference in absolute number of *S. pratensis* pollen grains does not appear to be related to population size (F2,28= 7.5, $p< 0.005$; table 7.4) . The absolute number of pollen grains differed also for *H. trivittatus* between the two populations it was sampled . The syrphid *E. tenax* could only be sampled at the site large-low.

Heterospecific pollen grains were from the types *Hieracium/ Hypochaeris/ Leontodon, Calluna/ Erica*, and *Achillea/ Tanacetum*, all species with pollen that could easily be picked up by the syrphids. Despite the fact that in the large *S. pratensis* population only one other species was in flower (*Potentilla erecta*), the loads of the insects contained between 22 and 80% heterospecific pollen grains. Most striking was the presence of pollen of *Erica/ Calluna*, species not present in the direct vicinity.

In summary, the average body load of syrphids contained respectively (from small and low diversity to large and high diversity of plants) 23, 29, 47 and 54 % *S. pratensis* pollen grains, and 95, 195, 53, and 238 absolute number of *S. pratensis* pollen grains (data derived from tables 7.3 & 7.4).

Plant observations

POLLEN COMPOSITION OF STIGMATIC LOADS

The mean proportion of conspecific (i.e. *S. pratensis*) pollen grains per flower head varied between 39 and 97% (fig. 7.5A). The proportion was positively related to population size and plant species richness (fig. 7.5D). In a curve-fit analysis, a logarithmic function gave the best description of the relation between population size and conspecific pollen (r^2= 0.47, $F_{1,58}$= 51.2, p< 0.001). The large/low population received a significantly higher proportion of *S. pratensis* pollen grains than the other populations ($F_{4,56}$= 19.2, p< 0.001=0.000, fig. 7.6A).

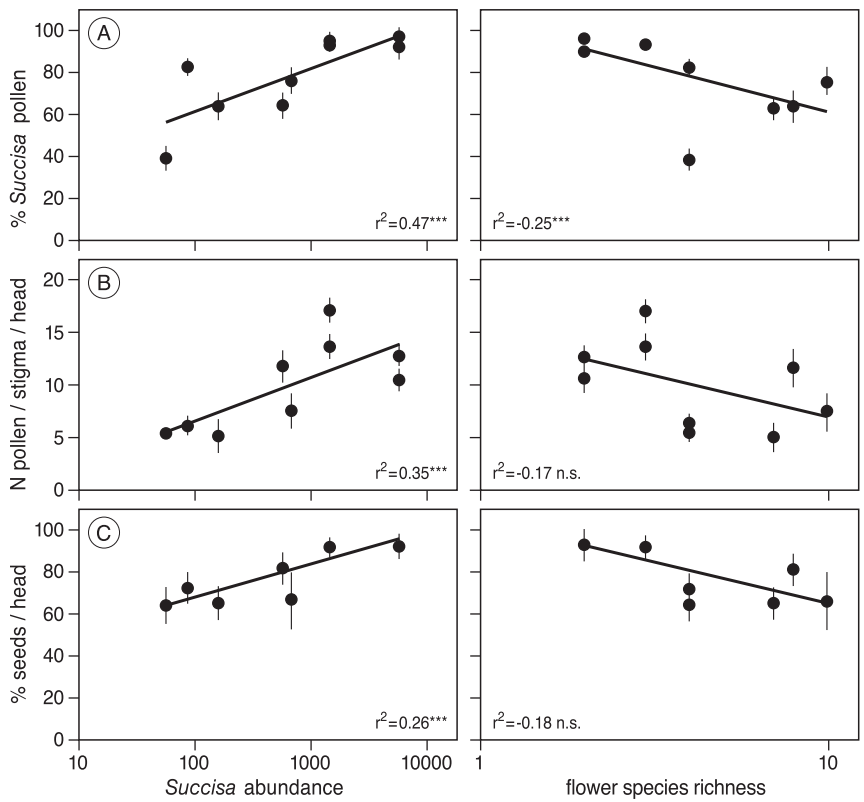

Fig. 7.5. Relation between *S. pratensis* "population size", i.e. flower abundance, or flower species richness (both log scaled) and the percentage of conspecific (*S. pratensis*) pollen on stigmas (A), the number of conspecific pollen grains deposited per stigma per head of *S. pratensis* (B) and potential seed set (C) in 2003. Potential seed set is based on a minimum of four pollen grains per stigma (see text).

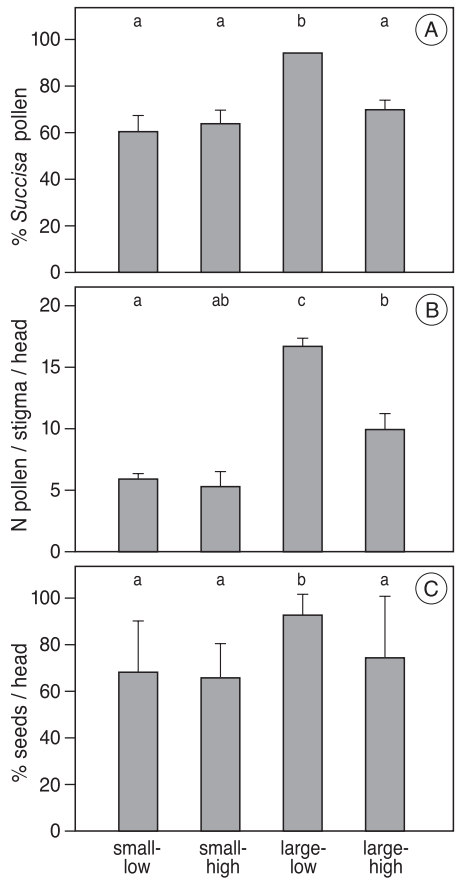

Fig. 7.6. Pollen composition of stigmatic loads in percentage of *Succisa pratensis* pollen (A), the number of conspecific pollen grains deposited per stigma per head of S. pratensis (B) and potential seed set (C) in populations different abundances of *S. pratensis* (small or large) and differing number of simultaneously flowering plant species (low or high) in 2003. Means are ±SE. Potential seed set is based on a minimum of four pollen grains per stigma (see text). Different letters indicate significant differences between populations.

Number of *Succisa* pollen per stigma

The mean number of *S. pratensis* pollen grains per stigma varied between 5 and 17 per stigma (fig. 7.5B). The number of pollen grains per stigma was positively related to population size, and negative, but less strongly, to plant species richness (fig. 7.5D). In a curve-fit analysis, logarithmic functions gave the best description of the relations (size: $r^2 = 0.35$, $F_{1,58} = 31.0$, $p < 0.001$; diversity: $r^2 = 0.25$, $F_{1,58} = 19.5$, $p < 0.001$). Generally, large populations had a larger number of *S. pratensis* pollen per stigma than small populations ($F_{4,56} = 18.8$, $p < 0.001$; fig. 7.5B). The large/low population received a significantly higher proportion of *S. pratensis* pollen grains than the other populations (fig. 7.6B).

Potential seed set

The mean percentage of flowers per head that potentially set seed varied between 65 and 96% (fig. 7.5C). The number of pollen grains per stigma was positively related to population size, but not plant species richness (fig. 7.5F). In a curve-fit analysis, a logarithmic function gave the best description of the relation between population size and the number of deposited pollen grains per stigma per head ($r^2 = 0.26$, $F_{1,58} = 20.3$, $p < 0.001$). Potential seed set was highest in the large population with a low plant diversity ($F_{4,56} = 8.4$, $p < 0.001$; fig 7.6C).

DISCUSSION

Insect species

Succisa pratensis populations in the Netherlands differed in the composition of the visitor guilds. Between 5 and 20 insect species per population were observed; in total (2002 and 2003 combined) 34 insect species were visitors of *S. pratensis*. In 2002, bumblebees were nearly absent: only 1.4% of the total number of visits were made by bumblebees. In 2003, bumblebees were responsible for 29.5% of the visits with locally more than 50%. These data illustrate that the composition of the visitor guilds may vary largely between populations and years; population size may have an effect but variation in flower visitor guilds is normal. Variation in flower-visiting insects was also found by various other authors (Herrera 1988, 1989 & 1995; Ashman & Stanton 1991; Eckhart 1995; Ramsey 1995; Fishbein & Venable 1996; Herrera 1996; Kato 1996; Traveset & Sáez 1997; Comba *et al*. 1999; Memmott 1999).

We never observed the bee species *Andrena marginata*. Knuth (1898) mentioned this bee species for *Knautia arvensis* (observations by Alfken and Höppner near Bremen, Germany), together with many other insect species, 57 species in total. Also Proctor *et al*. (1996) mentioned this bee species for *S. pratensis*. Knuth (1898) mentioned that bee species were up to 41% (solitary bees and bumblebees, but not *A. marginata*) and syrphids only 27% of the visiting species of *S. pratensis*. Adams (1955) observed 15 bee species and only three fly species on *S. pratensis* in Great Britain. Willis & Burkill (1895) presented 58 insect visitors of *S. pratensis*, but *A. marginata* was not among the Hymenoptera mentioned. Also in French *Succisa* populations (Vosges, Lorraine), *A. marginata* was not found (2 populations were studied in 2003, unpubl. data) and syrphids were the dominant flower visitors (between 50 and 95% of visits, and 51% of the species, N= 29 insect species). Bees, in total, brought up to 30% of the visits in one population, including small solitary bees (but not *A. marginata*).

Succisa pratensis is well known as food source for butterflies. Knuth (1898) mentioned 7 species of Lepidoptera, Jennersten (1984) mentioned four species that were responsible for 40% of the visits to *S. pratensis* heads. We observed six butterfly species (31.8% of all species) responsible for only 1.8% of the visits in 2002, and four butterfly species (17.4% of all species) responsible for 5.2% of the visits in 2003. The late flowering time may be one of the reasons for the absence of butterflies as visitors. In general the

frequency of visits of butterflies is low compared to other insect species visiting the same plant species. In addition, the number of pollen grains deposited on the stigma by butterflies is restricted (Jennersten 1984; Velterop 2000), with the exception of a few plant species in the northwest European flora.

The bulk of the flower visits to *S. pratensis* in the Netherlands was made by a restricted number of insect species, mainly syrphid species and the bumblebee *Bombus pascuorum*. In total for all populations together, in 2002 we found five syrphid species as the most frequent visitors: *E. horticola*, *H. trivittatus*, *H. pendulus*, *E. tenax* and *E. arbustorum/ nemorum* in order of decreasing abundance. In 2003, *E. tenax*, *H. trivittatus* and *B. pascuorum* were the most frequent species on *S. pratensis* (figure 7.2). The behaviour of these frequently occurring insect species determines pollination quantity and quality aspects of *S. pratensis*.

Small populations were visited by fewer insect species than large populations. In addition, small populations had sometimes bumblebees as most frequent visitor; in one large population bumblebees were the second frequent visitor. Taking into account not only the frequency of individuals, for instance the ratio between bumblebee and syrphids, but also *Succisa* pollen deposition (ratio bumblebees: syrphids = 1 : 0.7) the importance of bumblebees in relation to the number of deposited pollen increases (Kwak 1993). We expected that populations with many bumblebee visits should have a higher *Succisa* pollen deposition compared to heads in populations with the same visitation rates but visited by syrphids. Indeed, this expectation came true for the large populations in 2003.

Flower constancy in relation to the purity of the stigma pollen load

The behaviours of the most frequent insect species, syrphids in 2002, determined the pollen load on the stigmas. The flower constancy of syrphids is influenced by the population size of *S. pratensis* and the presence of co-occurring and simultaneously flowering plant species. Syrphid species differed in their flower constancy, measured simultaneously in one population, with *H. pendulus* being a less flower-constant species compared to *H. trivittatus* and *E. horticola*. Although individual syrphid species may be constant for the time that they were observed, they may carry a body pollen load with pollen grains of plant species not occurring in the direct vicinity. The load reflects the plant species that were visited during a longer period, preceding the foraging in a *S. pratensis* population. We found *Erica* and *Calluna* pollen, and the nearest population of these plant species is at least 300 m away from the *S. pratensis* population.

Population size of *S. pratensis* determines the purity of the body loads of insects. In small *S. pratensis* populations the average syrphid load contained between 23 and 18.5% *S. pratensis* pollen and in large populations between 46.5 and 54%, a clear effect of *S. pratensis* population size. The effect of the flowering environment is reflected in more heterospecific pollen deposition in a diverse environment, but only for *H. pendulus* regardless of the population size of *S. pratensis*. For the percentages of *S. pratensis* pollen on the stigmas we see the same trend: less conspecific pollen in smaller (around 60%) than in larger populations (70 and 100%).

Visitation rate and number of *S. pratensis* pollen per stigma and potential seed set

The visitation rate varied in 2002 between 5 and 17, in 2003 between 5 and 18 visits per *S. pratensis* head per hour. That means that every 3 to 12 minutes an insect landed on a *S. pratensis* flower head; this high visitation rate of *S. pratensis* led to the following comparison made by Lack (in Proctor *et al*. 1996): '…the number of visitors around each inflorescence may be so large that the scene resembles aircrafts stacked in the air waiting to land at a too-busy airport.' Assuming a visitation day of six hours, each head will have received between 30 and 108 visits. These values seem sufficient for complete pollination. However, to fulfil both the female and the male function of the flower (the flower head is in the male phase between 6-8 days, the female phase usually for only 1 day), also heads in the male phase need to be visited. One visit per day to a female head is not enough to pollinate all stigmas. To achieve full seed set, heads must be visited several times during the receptive female phase, which generally lasts only a single day. Each visitor spent such a short period per head, that this is too short to deposit at least four grains per stigma in all flowers of a head (Kwak 1993). Stigmas had received between 5 and 17 pollen grains at the end of the day (in 2003), but small populations had received significantly less conspecific pollen than stigmas in large populations. Potential seed set was high, and varied between 65 and 94% of the flowers per head per population. Small populations still do receive many insect visits, but the amount of available pollen in such small populations may be restricted. For instance, in 2003 the visitation rate in the small population Gasteren was around the same as in the large populations Reitma and Annen (10 visits per head per hour), but the resulting pollen deposition was respectively 6, 17 and 10 pollen grains per stigma. Thus, not only visitation rate is important for pollination, but also the number of conspecific pollen grains deposited. In small populations, flower density of the target species may be low and visitors may have lost the conspecific pollen before arriving on a *S. pratensis* head, for instance due to visits to other plant species.

In conclusion

Large populations of *S. pratensis* are visited by more insect species, but flowers in large *S. pratensis* populations do not receive more visits per head than flowers in small populations. However, *S. pratensis* flowers in large populations have a larger conspecific pollen load on the stigmas with the expectation that the potential seed set is also larger in large populations. The diversity of the environment, e.g. the number of co-occurring, simultaneously flowering plants, has no influence on the purity of the stigmatic pollen load: insects may forage for short periods on only one plant species (flower constant). However, they often carry a mixed body load sometimes containing pollen grains of plant species not occurring in the immediate vicinity.

Most of the data point in the same direction: there is no difference in visitation rates between small and large populations but the quality of pollination (number of *Succisa* pollen grains, purity of the stigmatic load, distribution of the pollen grains over the stigmas within a head) is more favourable for seed set in large than in small populations, with a strong effect of population size and much less of the diversity of the environment. This effect of diversity of the environment is pronounced in small *S. pratensis* populations. If

heterospecific pollen deposition leads to a lower seed set, then small populations in a diverse environment will have the lowest percentage of viable seeds per head and large populations in an environment with no other simultaneously flowering species will have the highest percentage of seed set. To date it is not known whether heterospecific pollen have a negative effect on the fertilisation of *S. pratensis*. The diversity of insects did not really play a role in the investigated populations. Only a few insect species are responsible for the bulk of the visits, but they all may visit also other plant species, resulting in heterospecific pollen deposition.

The bad quality of pollination in small *S. pratensis* populations decreases the reproduction, thereby increasing the extinction chance of these small populations. The presence of a specialist forager may increase the pollination quality. For *S. pratensis* this would be the bee *Andrena marginata*, but as we already know, it is extinct in the Netherlands now. The missing link of knowledge is the pollination quality of this bee: if it was better than syrphids and bumblebees, then the shift to a more generalist pollinator assemblage of only syrphids and bumblebees had negative consequences for the pollination of *S. pratensis*. On the other hand, it may be that even though *A. marginata* is a better pollinator than the other species, in large *S. pratensis* it does not matter as the pollination is also suffient with only generalist pollinators. Furthermore, small *S. pratensis* populations may be too small for sustaining *A. marginata* populations, and thus cannot benefit from this bee. Thus in the end there may be a positive feedback of declining plant populations affecting the abundance and the combined efficiency of all pollinator species, thereby speeding up the decline of the plant population. The presence and abundance of specialist pollinators therefore can be faster indicators of declining plant population size. Solitary bees are more affected by landscape deterioration than many other insect groups (chapter 3) and more than 50% of all bee species in the Netherlands are red-listed (Peeters & Reemer 2003). For many plant species this may be a "bad omen" if the same happens them as to *S. pratensis*, whose bee had disappeared long before the plant showed the steep decline.

Finally, the data presented in this chapter show how true the sentence of Fægri & van der Pijl (1979) is about flower visiting insects: ‘What they do today and in a particular place is not necessarily the same what they will do tomorrow and in another place'.